Applicant : Farb et al.
Serial No. : 09/652,345
Filed : August 31, 2000
Page : 2

Atto⬤'s Docket No.: 13594-010002

Error bars represent standard error. *Smooth curve* was determined by nonlinear regression using the logistic equation applied to pooled data. Fitted parameters are (GluR1) I_{max} =1.0, EC_{50}=27 μM, n_H=1.54; (GluR1 + PS) I_{max}=0.17, EC_{50}=23 μM, n_H=0.9; (GluR3) I_{max} =1.15, EC_{50}=27 μM, n_H=1.44; (GluR3 + PS) I_{max} =0.33, EC_{50}=32 μM, n_H=1.93; (GluR6) I_{max} =1.0, EC_{50}=550 nM, n_H=1.1; (GluR6 + PS) I_{max} =0.69, EC_{50}=570 nM, n_H=1.2. Figure 2F is a graph of data showing the concentration dependence of PS inhibition of recombinant GluR1 (O), GluR3 (□), and GluR6 (▲) receptors. Results are expressed as percentage change in the peak 100 μM (GluR1 and GluR3) or 10 μM (GluR6) kainate-induced current in the presence of PS. Each *data point* is the mean of three experiments; *error bars* indicate S.E.M. For GluR1 and GluR3, *smooth curves* are derived from fits to the Michaelis-Menten equation, as fits to the logistic equation yielded Hill coefficients close to 1, with no significant improvement in sum of squares (*F*-test, $P > 0.05$). Fitted parameters are (GluR1) EC_{50}=43 μM, E_{max} = -99%; (GluR3) EC_{50}=12 μM, E_{max} = -90%. For GluR6, the smooth curve is derived from a fit to the logistic equation, as Michaelis-Menten fits were significantly poorer (*F*-test, $P < 0.05$). Maximum inhibition was constrained to 100%, as an unconstrained fit yielded an extrapolated maximum inhibition >100%. Fitted parameters are EC_{50}=80 μM, n_H=0.29.--

Please replace the paragraph beginning at page 5, line 4, with the following rewritten paragraph:

--Figure 3 is a compilation of graphical representations of data which indicate that neuroactive steroids modulate NMDA responses of oocytes injected with specific NMDA receptor subunits. Figure 3(A) indicates the potentiation of the 100 μM NMDA response by PS in oocytes injected with $NR1_{100}$ + NR2A cRNA. The *solid bar* indicates the period of NMDA exposure; the *open bar* indicates the period of PS exposure. Figure 3(B) indicates inhibition of the 100 μM NMDA response by $3\alpha5\beta S$ in oocytes injected with $NR1_{100}$ + NR2A cRNA. The *solid bar* indicates the period of NMDA exposure; the *shaded bar* indicates the period of $3\alpha5\beta S$ exposure. Figure 3(C) indicates modulation of agonist efficacy by PS and $3\alpha5\beta S$ in oocytes injected with $NR1_{100}$ + NR2A cRNA. PS (100 μM) increases the NMDA I_{max} but does not affect the EC_{50}. $3\alpha5\beta S$ (100 μM) markedly reduces the NMDA I_{max} with little effect on EC_{50}. Peak

Applicant : Farb et al.
Serial No. : 09/652,345
Filed : August 31, 2000
Page : 3

Attorney's Docket No.: 13594-010002

NMDA responses are normalized to the peak 100 μM NMDA response. Each *data point* represents the mean of three experiments. *Error bars* represent standard error. *Smooth curves* are derived from fits to the logistic equation. Fitted parameters are (control) EC_{50}=29 μM, E_{max}=1.14, n_H=1.43; (+PS) EC_{50}=30 μM, E_{max}=3.21, n_H=1.54; (+3α5βS) EC_{50}=15 μM, E_{max}=0.35, n_H=1.66. Figure 3(D) is a graph indicating the concentration dependence of steroid modulation of the NMDA response of oocytes injected with $NR1_{100}$ + NR2A cRNA. NMDA (100 μM) and the indicated concentration of PS (●), 3β5βS (Δ), or 3α5βS ($\Box$) were applied simultaneously for 10 s. The peak NMDA-induced current is expressed relative to the average of control NMDA responses determined before application of steroid and after steroid washout. *Points* indicate mean of 6 (PS and 3α5βS), and 4 (3β5βS), experiments. *Error bars* indicate S.E.M. Smooth curves are derived from fits to the Michaelis-Menten equation, as fits to the logistic equation yielded Hill coefficients close to 1, with no significant improvement in sum of squares (*F*-test, $P > 0.05$). Fitted parameters are (for PS) EC_{50}=32 μM, E_{max}=4.43 (for 3α5βS) EC_{50}=41 μM, E_{max}=0.1; (for 3β5βS) EC_{50}=79 μM, E_{max}=0.26. (E) Concentration dependence for PS enhancement (●) and 3α5βS (Δ) and 3β5βS ($\Box$) inhibition of the NMDA response of oocytes injected with $NR1_{100}$ cRNA. NMDA (300 μM) and the indicated concentration of steroid were applied simultaneously. The peak NMDA-induced current is expressed relative to the average of control NMDA responses determined before application of steroid and after steroid washout. *Points* indicate mean of 6 (PS), 3 (3β5βS), and 3 (3α5βS) experiments. *Error bars* indicate S.E.M. Smooth curves are derived from fits to the Michaelis-Menten equation, as fits to the logistic equation yielded Hill coefficients close to 1, with no significant improvement in sum of squares (*F*-test, $P > 0.05$). Fitted parameters are (for PS) EC_{50}=26 μM, E_{max}=2.14; (for 3α5βS) EC_{50}=57 μM, E_{max}=0.02; (for 3β5βS) EC_{50}=144 μM, E_{max}=0.17.--

In the claims:

Please cancel claims 30 and 31 without prejudice.

All the pending claims currently under examination are reproduced below for the Examiner's convenience. Please amend claims 1 and 2 as follows: